Jurnal Teknologi

Full Paper

ANNOTATIONS AND DOMAIN ANALYSIS OF POLYKETIDE SYNTHASE GENES IN FUNGAL WHEAT PATHOGEN, *MYCOSPHAERELLA GRAMINICOLA*

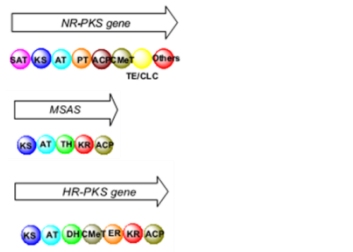
Rozida Mohd Khalid[a], Andy Bailey[b], R. J. Cox[c]

[a]School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia
[b]School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TH, United Kingdom
[c]School of Chemistry, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TH, United Kingdom

Article history
Received
18 September 2015
Received in revised form
29 September 2015
Accepted
15 October 2015

*Corresponding author
rozidakhalid@ukm.edu.my

Graphical abstract



Classification of PKS based on their structural architecture

Abstract

Mycosphaerella graminicola is an important pathogenic fungus of the wheat that causes *Septoria tritici* blotch. The genome of *M. graminicola* has been sequence and each domain of all polyketide synthase enzymes (PKS) was analysed and compared with known sequences from NCBI database. NCBI Basic Local Alignment Search Tool (BLAST) analysis and alignment using Vector NTi software suggested that *M. graminicola* has 10 PKS and 1 PKSNRPS (nonribosomal peptide synthetase) hybrid, which could be involved in pathogenicity. Out of the 10 polyketide synthase genes, two are non-reducing PKS (NRPKS) and eight are annotated as highly reducing PKS (HRPKS). The NRPKS was labelled as MgPKS1 and MgPKS9. The HR PKS were labelled as MgPKS2, MgPKS3, MgPKS4, MgPKS5, MgPKS6, MgPKS7, and MgPKS8. The PKS-NRPS also have a highly reducing PKS as part of the hybrid

Keywords: Mycosphaerella graminicola, Septoria leaf blotch, polyketide, polyketide synthase, polyketide-nonribosomal peptide synthetase

1.0 INTRODUCTION

Mycosphaerella graminicola strain IPO323 was fully sequenced by the Joint Genome Institute of the United States Department of Energy (JGI) [1]. *Mycosphaerella* is under the class Dothideomycete, and was suggested to be a model species by the JGI. One outstanding feature of Mycospaherella is that this fungus has a unique mechanism of infection compared with most sequenced fungi [2]. Instead of penetrating the host directly like *Magnaporthe grisea,* [3] *M. graminicola* infects *via* leaf stomata, suggesting no need of cell wall degrading enzymes. After the initial infection, the host does not show any symptoms of infection for 8 – 10 days despite the presence of a large quantity of fungal tissue. Under the right conditions, the mesophyll cells can suddenly undergo rapid collapse, resulting in chlorosis and necrosis. This collapse occurs without the presence of mycelium, probably due to production of soluble phytotoxins during the wheat-fungal interaction [4]. Thus, the use of bioinformatics analysis can be very useful in

prediction of pathogenesis and the production of phytotoxins in fungi. Phytotoxins usually derived from secondary metabolites like polyketides, which are well known to be potent phytotoxins in other *Mycosphaerella* species [5]. One example is juglone, a polyketide type phytotoxin which is produced by *Mycospaerella fijiensis*. Thus, it is feasible that *M. graminicola* produced phytotoxins derived from polyketide biosynthetic pathway.

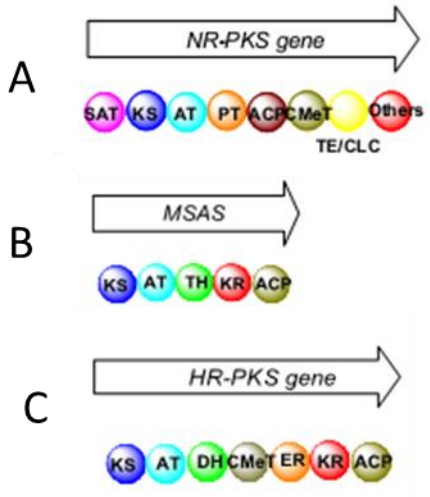


Figure 1 Classification of PKS based on their structural architecture, A = non-reducing PKS; B = partially-reducing PKS; C = highly-reducing PKS

Polyketide biosynthesis and the diverse structures of the polyketides produced depend on the polyketide synthase (PKS) enzymes. Each catalytic domain is linked together and each has specific functions. Examples of polyketides are the simple polyketide orsellinic acid and the highly complex T-toxin [6]. There are three main types of polyketides based on annotations and protein structures: type I, type II and type III. This study focused on Type I polyketide which consist of very large multifunctional proteins with covalently linked catalytic domains [7].

Type I PKS divided into two subtypes; modular and iterative systems [7]. Type I modular PKS are commonly found in bacteria and are usually very large enzymes. Type I iterative PKS are mostly found in fungi, and the enzymes are used repeatedly as needed [7]. The type I iterative PKS have been divided into three groups, based on the enzymatic domains that are present in the synthase which has different catalytic activities: non-reducing (NR), partially-reducing (PR) and highly-reducing (HR) enzymes [7], (Figure 1).

Non reducing PKS (NR-PKS) produce aromatic compounds, for example orsellinic acid, produced by the *Aspergillus* and *Penicillium* family and norsolorinic acid from *Aspergillus parasiticus* [8]. The general domain architecture of NR-PKS genes in fungi usually consists of a starter-unit ACP-transacylase (SAT), β-ketoacylsynthase (KS), acyltransferase (AT), product template (PT), acyl carrier protein (ACP) and thiolesterase/cyclases (TE/CLC) [9, 10]. Some NR-PKS appear to terminate after ACP, others contain methyltransferases and reductases [11].

Partially reducing PKS (PR-PKS) produce partial reduced compounds [12]. *Penicillium patulum* has MSAS protein that produces 6-methylsalicylic acid (6-MSA) and is the precursor of the mycotoxin patulin [13]. Genes that have been identified to encode MSAS are atX from *Aspergillus terreus* [14], pks2 from *Glarea lozoyensis* [12], 6msas from *Byssachlamys nivea* and aomsas from *Aspergillus westerdijkiae* [15]. The domain architecture of the PR PKS starts with the N-terminal KS, AT, thiolester hyrolase (TH), KR and ACP domains [12, 13].

The HR-PKS can control the formation of complex and highly reduced compounds like lovastatin, T-toxin, fumonisin B1, alternapyrone and squalestatin [16]. The domain structure of the HR-PKS are as follows; KS, AT, DH, C-MeT, ER, KR, ACP.

This study annotates and predicts the type of polyketides might be produced by polyketide synthase genes found in *Mycosphaerella graminicola*.

2.0 EXPERIMENTAL

All the PKS genes were translated into proteins *in silico*, and then aligned using the Vector NTi software [17]. The presence of introns were determined using Softberry tools [18], because up to now, there is no cDNA of a complete *M. graminicola* PKS gene. Multiple sequence alignments of amino acid sequences of PKS from *M. graminicola* and known PKS were conducted using Vector NTi according to similarity and homology, and the result was simplified by a phylogenetic tree. The domain boundaries of each PKS gene were determined by comparative analysis of the amino acid sequence with known and characterised PKS genes which were taken from the NCBI database, Broad Institute Fungal Genome Initiative database and Joint Genome Institute database. The known PKS genes are as stated in Table 1.

Table 1 List of known PKS used in this study

Organism (Acession number)	Gene	Protein	Domain	Final product
Aspergillus fumigatus ACJ13039	*alb1*	Ablb1p	SAT-KS-AT-PT-ACP-ACP-CLC/TE	YWA1
Aspergillus parasiticus Q12053	*pksL1/AflC/pksA*	NSAS	SAT-KS-AT-PT-ACP-CLC/TE	aflatoxin
Acremonium strictum CAN87161.2	*AsPKS*	MOS	SAT-KS-AT-PT-ACP-CMeT-R	xenovulene
Monascus purpereus BAD44749	*pksCT*	CitS	SAT-KS-AT-PT-ACP-CMeT-R	citrinin
Aspergillus terreus BAA20102.2	*atX*	MSAS	KS-AT-TH-KR-ACP	6-methyl salicylic acid
Glarea lozoyensis AAX35547.1	*pks2*	MSAS	KS-AT-TH-KR-ACP	6-methyl salicylic acid
Fusarium hetero-Sporum AAV66106	*eqiS*	EQS	KS-AT-DH-CMeT-(ER)-KR-ACP-NRPS	equisetin
Aspergillus terreus AAD39830	*lovB*	LNKS	KS-AT-DH-CMeT-(ER)-KR-ACP-C	lovastatin
Aspergillus terreus AAD39830	*lovF*	LDKS	KS-AT-DH-CMeT-ER-KR-ACP	lovastatin
Beauvaria bassiana CAL69597	*ORF4*	TENS	KS-AT-DH-CMeT-(ER)-KR-ACP-NRPS	tenellin
Phoma sp. AAO62426.1	*PhPKS1*	SQTKS	KS-AT-DH-CMeT-ER-KR-ACP	squale-statin

3.0 RESULTS AND DISCUSSION

3.1 Overall Analysis

Each PKS from *M. graminicola* was first compared with known PKS genes by multiple sequence alignment analysis and from the results, a phylogenetic tree was generated (Figure 2). The known PKS genes covered all three classes of fungal PKS: the non-reducing PKS (NR-PKS), partially-reducing PKS (PR-PKS) and highly-reducing PKS (HR-PKS). Each of these classes has characteristic domain arrangement and was grouped accordingly. Known domains such as β-ketosynthase, ACP, AT, KR, DH and ER domains are available in NCBI database, but domains such as SAT, PT and TH need to be examined manually using Vector NTi software. The clusters have grouped both known PKS and PKS from *M. graminicola* into roughly three groups: NR-PKS (yellow box), PR-PKS (blue box) and HR-PKS (pink box). Each of these classes has characteristic domain arrangement and was grouped accordingly. The sequences of known domains such as β-ketosynthase, ACP, AT, KR, DH and ER domains are available in NCBI database, but domains such as SAT, PT and TH need to be examined manually using Vector NTi software. Based on the grouping from Figure 2 and domain sequence pattern generated from NCBI and Vector NTi (data not shown), it was obvious that *M. graminicola* does not have any PR-PKS.

It can be proposed that MgPKS1 and MgPKS9 are most likely NR-PKS, because multiple sequence alignment showed highest homology towards NR-PKS, with annotation of SAT domain which is exclusive in NR-PKS. Figure 2 showed that these two PKS are grouped together along known NR-PKS such as AsPKS1 which encodes MOS for the production of 3-methylorcinaldehyde from *Acremonium strictum*.

Figure 2 also shows MgPKS1 protein being grouped with PKS genes which encode for THNS proteins such as PKS1 from *Nodulisporium* sp., pks1 from *Colletrotrichun lagenarium* and WdPKS1 from *Wangiella dermatitis*. This is also supported by multiple sequence alignment of the PT domain (details in domain analysis discussion), which was suggested to be involved in chain length mechanism [7].

MgPKS9 is grouped with tetraketide producing proteins such as MOS from *Acremonium strictum* which produces 3-methylorcinaldehyde [19] and CitS from *Monascus purpureus* which is involved in the production citrinin as the final product. Like MgPKS1, this data is supported by multiple sequence alignment of the PT domain (data not shown). MgPKS9 are most similar to AfoE from *Aspergillus nidulans* that involves in the production of a non reduced polyketide, asperfuranone [20].

MgPKS10 and MgPKSNRPS1 are all grouped together with known PKS with inactive ER such as ACE1 from *Magnaporthe grisea*, LNKS from *Aspergillus terreus* and TENS from *Beauvaria bassiana* [20]. Multiple sequence alignment and domain analysis of the ER domain from both MgPKS10 and MgPKSNRPS1 supported this result (data not shown).

The rest of the PKS from *M. graminicola* were grouped in the HR-PKS class, along with some known HR-PKS such as lovastatin diketide synthase (LDKS) and lovastatin nanoketide synthase (LNKS) from *Aspergillus terreus* (Figure 2) and supported by the multiple sequence alignment analysis (data not shown).

3.2 Domain Analysis

This report focused on the domain analysis of only 2 PKS, which is MgPKS1 (as NRPKS representative) and MgPKS2 (as HRPKS representative Three domains that are exclusive in NRPKS are SAT, PT and TE/CLC domains. The conserved catalytic sites of SAT are the FGDQ(S/T), the GXCXG sequence, and the conserved histidine residue (Table 2) [21]. The conserved catalytic site of the PT domain has the $GHXVXGX_5P_5$ motifs, while the TE/CLC domain has the GXSXG conserved motifs [22, 23].

In both PKS, analysis of the domain sequences using multiple sequence alignment analysis showed the highly conserved AT, KS and ACP domains, all with intact active sites. These domains are crucial for the transfer of acyl and condensation reaction of the growing ketide chain [7]. Mutations of the catalytic residue GPX_5TACSX (KS domain), GHSXG (AT domain) and XGXDSL (ACP domain) usually mean that the enzyme is inactive. Like some NRPKS, MgPKS1 has two ACP domains, similar to THNS from *Wangiella dermatitidis* that produces T4HN as well as *Aspergillus fumigatus* Ablb1p and *Aspergillus nidulans* WA that produce the compound YWA1. Both of the compounds are involved in melanin formation [22].

The PT domain analysis showed the $GHXVXGX_5P_5$ motifs, indicative of PT domain and the expected ACP motifs XGXDSL were present in both domains (Table 1). It was proposed that PT domain are involved in the chain length mechanism [7], so the PT sequence of the translated MgPKS1 was aligned with known PT domains: tetrahydroxynaphthalene (THNS) from *Colletotrichum lagenarium*, a pentaketide; CitS, from *Monascus purpureus* and ZS-B, both which are tetraketides and NSAS (octaketide). Multiple alignment analysis showed that MgPKS1 is grouped with THNS from *C. lagenarium*, suggesting that MgPKS1 encodes for a pentaketide, consistent with its possible role in melanin biosynthesis. The TE/CLC domain of MgPKS1 has all the required active site residues (Table 2), [23].

MgPKS2, being a highly reducing enzyme, has all the reducing enzymes motifs: KR, DH and ER. There is a conserved domain of KR, with the expected $SX_{12}YX_3(K/N)$ motifs.

Domain analysis of the ER sequence of MgPKS2 suggested that the ER domain is inactive due to mutations at the catalytic site, so that instead of the $GXGX_2(G/A)X_3(G/A)$ motifs, the gene has a SLGQALVTIA motif (Table 2). The mutation of glycine to serine is similar to the PKSF gene in *Alternia solani* [23]. Some PKS genes that have an inactive ER have a nearby trans-ER domain in the gene cluster, for example tenC in the production of tenellin and lovC in the production of lovastatin [20, 24]. However, MgPKS2 appears to have no nearby trans-ER. This suggests that the product produced by MgPKS2 protein would be absent of reduction of the double bonds, similar with aslanipyrone produced by PKSF and Fusarin C from *Gibberella moniliformis* [23, 25].

MgPKS2 also has a C-MeT domain, with a KVLEIGAGTA motif, but with a noticeable mutation at the glycine site which is replaced by alanine, suggesting that the methylation domain might not be functional (Table 2).

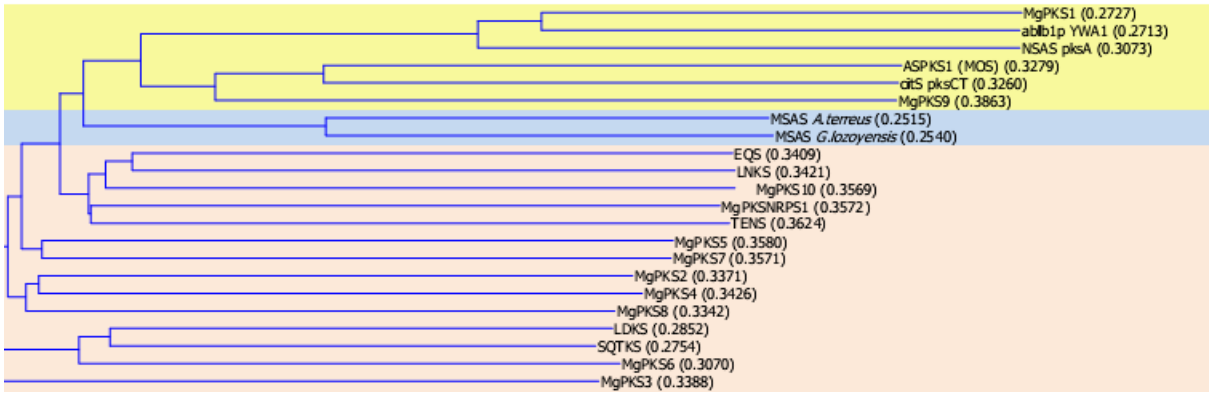


Figure 2 Phylogenetic tree of all PKS genes from M. *graminicola* compared with some known PKS genes. The yellow box indicates NR-PKS class, the blue box indicates PR-PKS class and the pink box indicates the HR-PKS class

Table 2 The conserved sequence of each catalytic domain in selected PKS from *M. graminicola*. MgPKS1 represents Non-Reducing PKS and MgPKS2 represents Highly Reducing PKS

Domain	Site description	Consensus	PKS Sequence	PKS
SAT	Active site glutamine Active site serine Active site histidine	FGD**Q**(S/T) GX**C**XG X**HX**	6-FQD**Q**T-12 112-GL**C**TG-118 241-**H**-243	MgPKS1
KS	Active site cysteine	GPX$_5$TA**C**SX	531-GPSYSIDTA**C**SS-544 174-GPSMTLDTA**C**SS-188	MgPKS1 MgPKS2
AT	Active site serine	GH**S**XG	985-GH**S**LG-991 672-GH**S**SG-678	MgPKS1 MgPKS2
DH	Dehydratase active site	**H**X$_3$GX$_4$P	1006-**H**VVRGSDVFP-1017	MgPKS2
C-MeT	SAM binding motif	(K/R)(I/V)(I/L)EIG(A/G)GTG	1486-KVLEIGAGTA-1497	MgPKS2
ER	NAD(P)H binding motif	GXGX$_2$(G/A)X$_3$(G/A)	2021-SLGQALVTIA-2032	MgPKS2
KR	Active site	**S**X$_{12}$**Y**X$_3$(**K**/**N**)	2362-**S**IAGLIGSPGQSN**Y**SSG**N**-2381	MgPKS2
PT	Conserved histidine	G**H**XVXGX5P$_5$	1319-G**H**MVNNAALCPS-1332	MgPKS1
ACP	Phosphopantheteine attachment site	XGXD**S**L	1699-GVD**S**L-1705 1834-GMD**S**L-1840 2554-YGVD**S**L-2561	MgPKS1 MgPKS2
TE/CLC	Active site serine, Histidine residue, Asp residue	GX**S**XG, X**H**X, X**D**X	1999-GW**S**QG-2005, 2067-**H**-2069, 2150-**H**-2152, 2028-**D**-2030	MgPKS1

4.0 CONCLUSION

Annotations of the PKS genes of *M. graminicola* gave two putative NR-PKS genes, eight putative HR-PKS and one truncated PKS-NRPS hybrid. The detail analysis of these genes can be used as a guide for heterologous expression studies.

Acknowledgement

The authors would like to thank the Ministry of Education Malaysia, Universiti Kebangsaan Malaysia and grant number GGPM-2013-033 for funding.

References

[1] http://genome.jgi.doe.gov/Mycgr3/Mycgr3.home.html.
[2] Encyclopaedia, H. W. D., http://www.hgca.com/hgca/wde/diseases/S.tritici/stricyc.html.
[3] Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M.; Kulkarni, R., Xu, J. R., Pan, H. Q., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomiets, E., Rehmeyer, C., Li, W. X., Harding, M., Kim, S., Lebrun, M. H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E., Birren, B. W. 2005. The Genome Sequence of the Rice Blast Fungus *Magnaporthe grisea*. *Nature*, 434 (7036), 980-986.
[4] S. B. Goodwin, S. B. M. B., B. Dhillon, A. Wittenberg, C. F. Crane, T. A. J. Van der Lee, J. Grimwood, A. Aerts, J. Antoniw, A. Bailey, B. Bluhm, J. Bowler, J. Bristow, P. Brokstein, B. Canto-Canche, A. Churchill, L. Conde-Ferraez, H. Cools, P. M. Coutinho, M. Csukai, P. Dehal, P. De Wit, B. Donzelli, A. J. Foster, K. Hammond-Kosack, J. Hane, B. Henrissat, A. Kilian, E. Koopmann, Y. Kourmpetis, A. Kuzniar, E. Lindquist, C. Meliepaard, N. Martins, R. Mehrabi, R. Oliver, A. Ponomarenko, J. Rudd, A. Salamov, J. Schwarz, H. Shapiro, I. Stergiopoulos, S. F. Torriani, H. Tu, R. de Vries, A. Wiebenga, L. Zwiers, I. V. Grigotiev, G. H. J. Kema. 2010. Finished Genome of *Mycosphaerela graminicola* Reveals Stealth Pathogenesis and Extreme Plasticity. Unpublished.
[5] El Hadrami, A. B., Kone, D., Lepoivre, P. 2005. Effect of Juglone on Active Oxygen Species and Antioxidant Enzymes in Susceptible and Partially Resistant Banana Cultivars to Black Leaf Streak Disease. *European Journal of Plant Pathology*. 113(3): 241-254.
[6] Schroeckh, V., Scherlach, K., Nutzmann, H. W., Shelest, E., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C., Brakhage, A. A. 2009. Intimate Bacterial-Fungal Interaction Triggers Biosynthesis of Archetypal Polyketides in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences of the United States of America* 2009. 106 34): 14558-14563.
[7] Cox, R. J. 2007. Polyketides, Proteins and Genes in Fungi: Programmed Nano-Machines Begin to Reveal Their

Secrets. *Organic & Biomolecular Chemistry*. 5(13): 2010-2026.
[8] Awakawa, T., Yokota, K., Funa, N., Doi, F., Mori, N., Watanabe, H. and Horinouchi, S. 2009. Physically Discrete Beta-Lactamase-Type Thioesterase Catalyzes Product Release in Atrochrysone Synthesis by Iterative Type I Polyketide Synthase. *Chemistry & Biology*. 16(6): 613-623.
[9] Crawford, J. M., Korman, T. P., Labonte, J. W., Vagstad, A. L., Hill, E. A., Kamari-Bidkorpeh, O., Tsai, S. C., Townsend, C. A. 2009. Structural Basis for Biosynthetic Programming of Fungal Aromatic Polyketide Cyclization. *Nature*. 461(7267): 1139-U243.
[10] Minto, R. E. and Townsend, C. A. 1997. Enzymology and Molecular Biology of Aflatoxin Biosynthesis. *Chemical Review*. 97(7): 2537-2555.
[11] Crawford, J. M. and Townsend, C. A. 2010. New Insights Into the Formation of Fungal Aromatic Polyketides. *Nat Rev Microbiology*. 8(12): 879-89.
[12] Beck, J., Ripka, S., Siegner, A., Schiltz, E. and Schweizer, E. 1990. The Multifunctional 6-methylsalicylic Acid Synthase Gene of *Penicillium patulum*-It's Gene Structure Relative to that of Other Polyketide Synthases. *European Journal of Biochemistry*. 192(2): 487-498.
[13] Puel, O., Tadrist, S., Delaforge, M., Oswald, I. P. and Lebrihi, A. 2007. The Inability of *Blyssochlamys Fulva* to Produce Patulin is Related to Absence of 6-Methylsalicylic Acid Synthase and Isoepoxydon Dehydrogenase Genes. *International Journal of Food Microbiology*. 115(2): 131-139.
[14] Fujii, I., Ono, Y., Tada, H., Gomi, K., Ebizuka, Y., Sankawa, U. 1996. Cloning of the Polyketide Synthase Gene atX from *Aspergillus terreus* and Its Identification as the 6-Methylsalicylic Acid Synthase Gene by Heterologous Expression. *Molecular & General Genetics*. 253(1-2): 1-10.
[15] Bacha, N., Dao, H. P., Atoui, A., Mathieu, F., O'Callaghan, J., Puel, O., Liboz, T., Dobson, A. D. W. and Lebrihi, A. 2009. Cloning and Characterization of Novel Methylsalicylic Acid Synthase Gene Involved in the Biosynthesis of Isoasperlactone and Asperlactone in *Aspergillus westerdijkiae*. *Fungal Genetics and Biology*. 46(10): 742-749.
[16] Chiang, Y. M., Szewczyk, E., Davidson, A. D., Keller, N., Oakley, B. R. and Wang, C. C. C. 2009. A Gene Cluster Containing Two Fungal Polyketide Synthases Encodes the Biosynthetic Pathway for a Polyketide, asperfuranone, in *Aspergillus nidulans*. *Journal of the American Chemical Society*. 131(8): 2965-2970.
[17] https://www.lifetechnologies.com/VectorNTI.
[18] http://linux1.softberry.com/berry.phtml?topic=fgenesh-m&group=programs&subgroup=gfind.
[19] Bailey, A. M., Cox, R. J., Harley, K., Lazarus, C. M., Simpson, T. J. and Skellam, E. 2007. Characterisation of 3-methylorcinaldehyde Synthase (MOS) in *Acremonium strictum*: First Observation of a Reductive Release Mechanism During Polyketide Biosynthesis. *Chemical Communications*. 4053-4055.
[20] Halo, L. M., Marshall, J. W., Yakasai, A. A., Song, Z., Butts, C. P., Crump, M. P., Heneghan, M., Bailey, A. M., Simpson, T. J., Lazarus, C. M. and Cox, R. J. 2008. Authentic Heterologous Expression of the Tenellin Iterative Polyketide Synthase Nonribosomal Peptide Synthetase Requires Coexpression with an Enoyl Reductase. *Chembiochem*. 9(4): 585-594
[21] Crawford, J. M., Dancy, B. C. R., Hill, E. A., Udwary, D. W., Townsend, C. A. 2006. Identification of a Starter Unit Acyl-Carrier Protein Transacylase Domain in an Iterative Type I Polyketide Synthase. *Proceedings of the National Academy of Sciences of the United States of America*. 103(45): 16728-16733.
[22] Watanabe, A., Fujii, I., Sankawa, U., Mayorga, M. E., Timberlake, W. E., Ebizuka, Y. 1999. Re-identification of *Aspergillus nidulans* wA Gene to Code for a Polyketide Synthase of Naphthopyrone. *Tetrahedron Letters*. 40(1): 91-94.
[23] Kasahara, K., Fujii, I., Oikawa, H. and Ebizuka, Y. 2006. Expression of *Alternaria solani* PKSF Generates a Set of Complex Reduced-Type Polyketides with Different Carbon-Lengths and Cyclization. *Chembiochem*. 7(6): 920-924.
[24] Kennedy, J., Auclair, K., Kendrew, S. G., Park, C., Vederas, J. C., Hutchinson, C. R. 1999. Modulation of Polyketide Synthase Activity by Accessory Proteins During Lovastatin Biosynthesis. *Science*. 284(5418): 1368-1372.
[25] Song, Z. S., Cox, R. J., Lazarus, C. M. and Simpson, T. J. 2004. Fusarin C Biosynthesis in *Fusarium moniliforme* and *Fusarium venenatum*. *Chembiochem*. 5(9): 1196-1203.